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Review Article

Genetic Diversity, Zoonotic Risk and "One Health" Initiative of Bovine Brucellosis

Riasat Wasee Ullah[1,3], Jafir Hussain Shirazi[1], Muhammad Abubakar[2], Aamer Bin Zahur[3], Asma Latif[3], Tahseen Alam[1]

[1]Department of Microbiology, Quaid-i-Azam University Islamabad, Pakistan; [2]National Veterinary Laboratories, Park Road Islamabad, Pakistan; [3]Animal Health Program, Animal Science Institute, National Agricultural Research Centre, Park Road, Islamabad, Pakistan
*Corresponding author: riasatwasee252@yahoo.com

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ABSTRACT

Brucella is one of the world major Zoonotic pathogen which is responsible for great economic losses and causes human morbidity. Especially in those countries which are based on agricultural and livestock where spread of this pathogen is very easy to human beings. The main objective of this review is to emphasis on new molecular techniques and genetic diversity of *Brucella* species which can be used to understand the pathogenicity and virulence of this pathogen and about "One Health" initiative umbrella where respective countries may plan to address zoonotic diseases like brucellosis which causes severe losses in livestock and public health economy. Out of ten species of *Brucella* five species are of zoonotic concern. The most pathogenic species for human is *B. melitensis, B. melitensis, B. abortus and B. suis* which are also potential bio-weapons. Transmission from person-to-person is rare, so infection is not spread from infected persons. Consequently, control and eradication of the brucellosis from the natural animal reservoirs leads to decrease in the incidence of human infection. It is evident that regardless of pathogenicity of the different species of the *Brucella*, the genus has genetic similarities with in species. There is high level of nucleotide similarities between *Brucella* species, but vary in host tropism and pathogenicity. "One Health" initiative is a new concept for the control of emerging zoonotic diseases. In which the professionals from different professions like Veterinary profession, Medical Profession, Wild life and social communities work together for one purpose. Especially in developing countries where test and slaughter method cannot be implemented for the control and eradication of animal diseases. One Health Initiative should be started by these countries to control and eradicate the zoonotic diseases and ultimately wellbeing of humanity.

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INTRODUCTION

Bovine brucellosis is one of the most important Bacterial diseases affecting cattle (Corbel, 1997) The causative agents of this disease is *Brucella abortus* and with an emerging importance of *B. melitensis* are zoonotic pathogens that can cause a severe disease in humans, therefore highlighting the importance of its control (OIE, 2009). A wide range of animals is affected by the Brucellosis infection for example buffaloes, goats, sheep, camels, pigs and reindeers etc. other mammals are also affected but less frequently as compared to aforesaid cattle (Charters, 1980). Brucellosis is characterized by abortion, with excretion of the organisms in uterine discharge and in milk. Major economic losses result from abortion, loss of calves, and reduced milk yield in females and infertility in males and it can be diagnosed on the basis of abortion and discharged contents in milk and as well as uterine discharge (WHO, 1971). Brucellosis is a zoonotic infection and a solemn jeopardy to public health (Lapaque et al., 2005). Bovine brucellosis is occurring worldwide except where eradication program worked (Seleem et al., 2010). Entry sites for *Brucella* are all those sites which are in continuous directly exposed to the different pathogens. Although *Brucella* may enter the body by either route but major routes of entry are lungs, digestive tract, skin and mucosal layers etc. inspite of these routes *Brucella* may cause local infections by entering through blood and lymph. By entering through these routes it infects the tissue and causes local tissue damage (Lapaque *et al*., 2005).

Testing of livestock for brucellosis is done by culture and serology or by testing milk samples (Nielsen, 2002).The main serological test used for diagnosis of brucellosis is the Rose Bengal Plate agglutination Test (RBPT), which has very high (>99%) sensitivity but low specificity (Barroso *et al*., 2002). As a result, the positive predictive value of this test is low and a positive result is required to be confirmed by some other more specific test like serum agglutination test (SAT) and ELISA (Memish et al., 2002). With the advancement of molecular techniques it is now described that six species of *Brucella* contain almost > 90 % homology with each other. (Whatmore, 2009).

TAXONOMY OF THE *BRUCELLA*

Classification of genus *Brucella* has passed through different phases. Until 1985 the genus *Brucella* was classified into six species. But later on this classification was aborted and all the species were placed under one species. (Verger et al., 1985) But

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again in 2003 the committee on *Brucella* taxonomy agreed to reinstate the classification of 1985 and divided the *Brucella* into six species. (Oysterman and Moriyon, 2006) In 2007 *Brucella ceti* and *Brucella pinnipedialis* (infecting preferentially cetaceans and pinnipeds, respectively) were recognized as new species (Foster et al., 2007) In 2008, another new species of *Brucella* was first isolated in the common vole (Microtus arvalis) ,it was recognized as *Brucella microti*. (Scholz et al., 2008b) recently *Brucella inopinata* was isolated from a breast implant infection in an elderly woman with clinical signs of brucellosis. (Scholz et al., 2010) Recent reports describes isolation and characterization of strains from marine mammals which are known as *Brucellae* however characteristics are not similar with already recognized *Brucella* species. (Clavareau et al., 1998, Cloeckaert et al., 2001, Jahans et al., 1997) According to current taxonomic classification there are 10 species of the *Brucella* genus in total which are recognized.

INVADING THE IMMUNE SYSTEM

Brucella is facultative intracellular organism which survives within the host cell and bypasses the host normal immune system (Ko¨ hler et al., 2002, 2003; Baldwin and Goenka, 2006) *Brucella* species at first infect the phagocytes. and in these phagocytic cells it can resist many stresses. And eventually reach its replication site i.e. placental trophoblasts and here it replicates extensively. (Kohler et al., 2002).It modulates different immune cell functions. Such as in dendritic cells it interferes with their antigen processing and also interferes in their maturation. (Roop et al., 2009). It can also prolong the survival rate and duration of immune cells. for example in macrophages it prevents apoptosis and long term survival in the reticluendothelial system. (Gorvel and Moreno, 2002) After bypassing the immune system *Brucella* reaches its replication site and it focus on its replication. During pregnancy uterus is the area which is immune privileged area. Here once *Brucella* reaches it extensively replicates and causes abortion ultimately. (Neta et al., 2010)

GENETIC DIVERSITY OF *BRUCELLA*

Different genetic combinations present in a genetic pool is called as genetic biodiversity. Due to the importance of genetic diversity studies most of the researchers are now focused not only on the identification of the new markers but developing and designing suitable techniques for the discrimination between the genus currently under discussion to facilitate the government bodies. Which in turn devise and plan for epidemiological surveys and also for control and eradication programs against this disease in their respective countries. It is evident from aforementioned discussion that *Brucella* although comprises of different species but these species have similarity in their genomic make up.

It is evident that regardless of pathogenicity of the different species of the *Brucella*, the genus has genetic similarities with in species. (Whatmore, 2009) There is high level of nucleotide similarities between *Brucella* species, but vary in host tropism and pathogenicity. Now a day's techniques are being developed to assess the genetic biodiversity of *Brucella*. (Vizcaı´no et al., 2000; Morenoet al., 2002; Bricker, 2002) It is clear with the help of molecular techniques that there is high degree of identity between the DNA of classical *Brucella* species. On the bases of DNA-DNA hybridization techniques, More than 90 percent similarities are present among all species of *Brucella*. (Hoyer and McCullough, 1968a, 1968b; Verger et al., 1985).

POPULATION GENETICS OF *BRUCELLA*

There are different tools which are used to study genetic diversity. One of the techniques used for such type of study is Multi Locus Enzyme Electrophoresis (MLEE) technique (Whatmore et al., 2007). It has been set as a gold standard for population genetic studies and molecular epidemiological studies of pathogenic bacteria. It is important to measures the genetic diversity of the bacterial pathogen, the virulence factors and susceptibility of the microbes towards antibiotics can be a helpful tool for epidemiological studies. There is little evidence that bacterial population studies can be applied to *Brucella*. By using multilocus enzyme electrophoresis (MLEE) techniques population genetics has been studies for *Brucella*. (Boerlin, 1997) Some of the assays are suitable for gene level study of *Brucella*i.e.Pulsed-field gel electrophoresis, Insertion sequence based typing, PCR typing, Restriction fraction length polymorphism based approaches, Shift to 'genome-based' typing approaches, Tandem repeat based typing, Multilocus sequencing and SNP typing.

COMPARISON OF GENOMICS

The availability of gene sequencing methods and techniques are helpful to understand about the pathogenicity and biological changes in the group under comparative study. In other words it can be easy to compare genome as well as other features of group members by the use of advanced research techniques. In 2002 *B. melitensis* 16 M genome was sequenced successfully. Following this genome sequence was successfully done for other strain of *Brucella* i.e. *B. Sui* 1330. (DelVecchio et al., 2002; Paulsen et al., 2002). After comparison of genome of these two strains it was found that their genomic make up is 98-100% identical. After a gap of almost three years third strain of *Brucella*'s genome was sequenced. i.e. B. abortus genome sequence was published in 2005. (Halling et al., 2005).

MULTILOCUS ENZYME ELECTROPHORESIS (MLEE)

It is revealed that all three genomes are very similar with each other. These genomic studies helped in understanding the pathogenicity. (Halling et al., 2005; Chain et al., 2005) An extensive study was done on genomic similarity and dissimilarity of these three strains fully seuquenced genomes. After Examination of 2308 sequences it was found more than hundred base pairs are unique to B.melitensis and B suis. But these unique base pair sequences are also found in B. abortus.Later this was confirmed that B.abortus is most closely related to B.melitensis. (Chain et al., 2005) Recently a full sequence of vaccine strain of B.abortus S19 was published. (Crasta et al., 2008) Multilocus enzyme electrophoresis (MLEE) is standard method to assess the genetic diversity of the bacterial genome. If genetic distance is larger than 0.5 than recognize as new species. MLEE of 99 isolate of *Brucella* revealed that there is very limited genetic diversity in this group. (Gandara et al., 2001)

ZOONOTIC RISK

Brucellosis is the most important zoonotic disease worldwide and it is responsible for huge economic losses affecting livestock and human population. In most of the countries this disease is endemic. (Godfroid et al., 2005) Control of this disease is depends upon the rapid detection method which can also applied in field. DNA based detection methods are now developed to diagnose this disease. After the release of full genome of *Brucella* it is now easy to understand the virulence and pathogenesis of this disease.

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TRANSMISSION

There are many factors which are responsible for transmission of the disease in livestock as well as in humans. And it is varies with geographically conditions, climate, age, sex and species. (Gul and Khan, 2007). In a study conducted by Abubakar et al. (2010) showed that incidence of the disease is increased with age and also increased in sexually matured animals. Brucellosis is transmitted in human by different means e,g direct animal contact, inhalation, consumption of unpasteurized milk and other dairy product and undercooked meat products. (Malik GM,. 1997) *Brucella* can survive for long period of time in dung, water, dust, soil, aborted fetuses, meat and dairy products. And it is occupational risk for human, veterinarians and other related personals because of very low infectious dose. (Smits and Cutler, 2004) In female animals, it can reside in udder and secrete in milk and in male animals epididymitis and orchitis can lead to temporary or permanent infertility. The increase in travel from endemic to non-endemic area can increase the importance and eradication of this disease. (Corbel, 2006) Worldwide more than 500,000 humans are affected with brucellosis. (Pappas et al., 2006)

ZOONOTIC IMPACT

Five out of nine species of *Brucella* can infect humans in which most pathogenic for human is *B. melitensis*. And followed in descending order by *B. suis*, *B. abortus* and *B. canis* (Acha et al., 2003). In last few years the zoonotic characteristics of the marine *Brucella*e (*B. ceti*) has also been reported. (Brew et al., 1999; McDonald et al., 2006; Sohn et al., 2003). *B. melitensis*, *B. suis* and *B. abortus* are listed as potential bio-weapons by the Centers for Disease Control and Prevention because *Brucella* species are highly infectious in nature and can be easily aerosolized. Transmission typically occurs directly with skin lesion, inhalation of aerosols and consuming contaminated or unpasteurized dairy and milk products. (Young, 1998; Christopher *et al*., 2010). Due to resemblance of *Brucella* symptoms with influenza it is difficult to detect the outbreak. (Chain et al., 2005). Studies in the sub Saharan Africa suggest that cattle are the main source of the *Brucella* spp. And the *Brucella abortus* infected cattle are the main source of the causing disease in humans in sub- Saharan Africa. (McDermott and Arimi, 2002).

WORLDWIDE DISTRIBUTION AND ECONOMIC IMPACT

Worldwide human brucellosis prevalence has been studied. The high risk countries are south and Central America, Eastern Europe, Asia, Africa, Mediterranean Basin, the incidence of disease in the Eastern Mediterranean Region ranges from 1 per 100,000 to 20 per 100,000 populations. Brucellosis is endemic in Saudi Arabia, where the national sero-prevalence is 15% (Memish, 2001). The geographical distribution is constantly changes with new emerging and re-emerging centers. Brucellosis causes more than 500,000 human cases worldwide. This disease has a very limited geographical distribution but still have a noteable status in the western Asia, latin America and some part of Africa. (Seleem et al., 2010).

The countries like UK, Sweden, Netherlands, Canada, Cyprus, Denmark, Finland, New Zealand and Norway. The Eastern and northern Asia, Central Asia and central South America are still not free from brucellosis. In some countries the *B. melitensis* has never been reported. According to (Robinson, 2003), there are no reliable reports that *B. melitensis* is eradicated from small ruminants from any country. Due to some socioeconomic, sanitary and political issues brucellosis is still more prevalent in some part of the world. Even made advances in surveillance techniques. (Pappas et al., 2006) The disease cause huge economic losses in animal production system in the form of abortion, reduced milk yield and delayed conception and in public health in the form of cost of treatment and productivity loss. Annually 600 Million US doller losses are due to brucellosis in Latin America. The US national brucellosis eradication program, while costing $3.5 billion between 1934 and 1997, the cost of reduced milk production and abortion in 1952 alone was $400 million (Acha et al., 2003; Sriranganathan et al., 2009).

MEASURES FOR CONTROL: ESPECIALLY IN DEVELOPING COUNTRIES

Brucellosis is highly infectious and contagious with rapid inter and intra-herd spreading (Ahmad, 2005). The main important objective to control of this disease is reducing the impact of this disease on animal health and especially human concerns. An effective control measures include the surveillance of the infected herds with cost effective and more specific diagnostic tests (Abubakar et al., 2012). Separation of the infected herd from healthy ones and eradicate the reservoirs to protect the susceptible population in that area. Vaccination of the domestic animals and wild life reduced the risk for human health.

Alexander et al., (2012) reported that the areas where buffaloes are present it is more likely that these species are may be the important in the transmission of the disease. And it is recommended that wild life species are also included in the surveillance studies. According to (Saleem M.N et all 2009) said that cost effective control measures for brucellosis is known but problem is lack of funding and awareness in respective authorities.

It is difficult to implement control strategies in developing countries considerable efforts have been required for the infrastructure and for awareness campaigns about *Brucella* risks Providence Laboratory facilities and trained personals for collection and testing of samples for regular surveillance activities. So these are the challenges which are facing by the developing countries. By controlling disease in animals may reduce the risks in humans considerably. And its happen in developed countries where bovine brucellosis is controlled or eradicated the human risk becomes low. So for the control of the disease there should be a strong support by the concerning government. The farmers, governments, milk industry and consumers must work together for the control of this disease.

Three types of control measures are described by the Abubakar et al., (2012) which are first: to eliminate the reservoir by proper quarantine and hygiene measures. Second: reduced or breaking the connection between reservoir and susceptible population. Third: by immunization with quality vaccines.

ONE HEALTH INITIATIVE AND BRUCELLOSIS

One health initiatives although is very successful for emerging zoonotic diseases but for brucellosis there is lake of conceptual frame work for the control of brucellosis. In developing countries there is no one health surveillance and control system for zoonotic disease. By implementing these initiatives for the control of the disease ultimately there are benefits for Public health and concerning societies. (Zinsstag et al., 2007)

Coker et al, (2011) said and it is obviously true that changes are occurs in the livestock sector and due to re-emerging of zoonotic diseases a new one heath research and policies has to be defined. The most important thing is the correct diagnosis for the disease both in Humans and Livestock due to zoonotic pathogen. And assure that what type of species is involved in human and in livestock. This approach leads to the proper planning of the surveillance and control of the disease under one health initiatives. According to Jones et al.,

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(2008), almost two third of the human pathogens are zoonotic and are of great concern because of causing deadly disease in humans. So it is important to have a global surveillance and control system for the emerging zoonotic diseases like brucellosis.

In trading live animals the OIE emphasizing on avoid transmission of the diseases. So it is important to have a worldwide standardized detecting system. The OIE also prescribed tests which are implicated in the field conditions and these are also appropriate in the developing countries. But the important thing is that rather emphasizing on detection systems it is important to detect the reservoir animals in the population from where disease occurs (Godfroid et al. 2012).

"One Heath" initiative is only successful when all related professionals work together like veterinarians, medical personals, and wild life professionals It will play a major role in the control of zoonotic diseases like brucellosis.

CONCLUSION

Brucella is a zoonotic pathogen which causes heavy losses in livestock as well as in public health. Developed world eradicate this disease by Test and Slaughtered method but this method is not suitable for developing countries, so to addressed these challenges it is important to control the disease in Livestock ultimately reduced risk for public health and obviously in livestock also. Developing countries or countries where this disease is endemic must be seriously planned and make strategies for the control and eradication of this disease. Now a day "One Health" Concept is being introduced which is very helpful in combating different zoonotic disease. After the discovery of *Brucella* Genome it is now easy to understand the virulence of this bacterium and how it causes the disease. And it may helpful in new diagnostic techniques for brucellosis and host specificity determination in future. New virulence factors are also recognized with the help of genetic techniques. After the above discussion it is recommended that "One Health" initiative should be started in developing countries which are in process to combat with this disease and also for other endemic zoonotic diseases. So in the "One Health" umbrella one should know his responsibilities and make strategies for control and eradication if zoonotic diseases that is suitable for each country and all professional work together for the well-being of humanity.

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